

Stereological comparison of 3D spatial relationships involving villi and intervillous pores in human placentas from control and diabetic pregnancies

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ABSTRACT

In human placenta, 3D spatial relationships between villi and the maternal vascular bed determine intervillous porosity and this, in turn, influences haemodynamics and transport. Recently-developed stereological methods were applied in order to examine and quantify these relationships. Placentas were collected after 37 wk from control pregnancies and those associated with maternal diabetes mellitus classified according to duration and severity (White classification scheme). Two principal questions were addressed: (1) are normal spatial arrangements maintained in well-controlled diabetes mellitus? and (2) do arrangements vary between diabetic groups? To answer these questions, tissue sections cut at random positions and orientations were generated by systematic sampling procedures. Volume densities of villi (terminal + intermediate), intervillous spaces and perivillous fibrin-type fibrinoid deposits were estimated by test point counting and converted to global volumes after multiplying by placental volumes. Design-based estimates of the sizes (volume- and surface-weighted volumes) of intervillous 'pores' were obtained by measuring the lengths of point- and intersection-sampled intercepts. From these, theoretical numbers of pores were calculated. Model-based estimates (cylinder model) of the hydraulic diameters and lengths of pores were also made. Second-order stereology was used to examine spatial relationships within and between villi and pores and to test whether pair correlation functions deviated from the value expected for 'random' arrangements. Estimated quantities did not differ significantly between diabetic groups but did display some departures from control values in non-insulin-dependent (type 2) diabetic placentas. These findings support earlier studies which indicate that essentially normal microscopical morphology is preserved in placentas from diabetic subjects with good glycaemic control. Therefore, it is likely that fetal hypoxia associated with maternal diabetes mellitus is due to metabolic disturbances rather than abnormalities in the quantities or arrangements of maternal vascular spaces.

Key words: Diabetes mellitus; pregnancy; placenta.

INTRODUCTION

In diabetic pregnancies, the fetus is exposed to increased risk of morbidity and mortality, metabolic abnormalities (including hyperglycaemia) and hypoxic stress (Desoye & Shafir, 1996; Hadden, 1996; Casson et al. 1997). Some of these problems might be mediated by changes in placental morphology but morphometric studies have been inconsistent about the nature and extent of such changes (Aherne & Dunnill, 1966; Jones & Fox, 1976; Haust,

1981; Singer et al. 1981; Teasdale, 1981, 1983, 1985; Björk & Persson, 1984; Boyd et al. 1986; Teasdale & Jean-Jacques, 1986; Stoz et al. 1988). The inconsistencies might be attributable to various factors including arbitrary tissue sampling, model-based morphometry, failure to cater for various confounders (e.g. mode of delivery, sex of neonate, duration and severity of diabetes mellitus) and mixing patients differing in duration, severity and quality of glycaemic control. Recent stereological studies conducted on random tissue samples from defined groups of well-

controlled diabetic patients have shown that changes mainly affect the downstream (fetal) side of the placenta (Mayhew et al. 1994; Mayhew & Sisley, 1998) and lead to improvements in transplacental oxygen transport measured as morphometric diffusive conductances (Mayhew et al. 1993).

Despite apparent normality of structural quantities on the maternal side, it is possible that differences in the spatiotemporal relationships between villi and the intervillous space occur in diabetic placentas. For example, it has been shown that villous surface does not expand isomorphically with villous volume (Mayhew, 1996). These relationships could affect the porosity (Schmid-Schönbein, 1988) of the intervillous space through which maternal blood percolates and, thereby, affect placental transport and haemodynamics. Indeed, there is evidence that uteroplacental blood flows are reduced in diabetic pregnancies and influenced by levels of maternal hyperglycaemia (Nylund et al. 1982). Other things being equal, a more porous intervillous space is expected to conduct maternal blood more easily.

Recent developments in design-based stereology make it possible to monitor relationships between villi and intervillous spaces, including pore sizes, in new ways. For instance, villi and intervillous pores have been studied successfully in placentas from normal and abnormal pregnancies by estimating their volume-weighted star volumes (Karimu & Burton, 1993; Mayhew & Wadrop, 1994; Lee & Mayhew, 1995). Until recently, this volume (Gundersen & Jensen, 1985) was the only available measure of size for arbitrary 3D spaces making it a potentially valuable quantitative tool for determining the volumes of the complexly-interconnected pores of the intervillous space and the ramifications of villous trees. However, it is now possible to estimate a surface-weighted star volume (Reed & Howard, 1998) and so express pore size in a way which is sensitive to changes in villous surface area. Finally, 3D spatial relationships within and between villi and intervillous pores can be defined using second-order stereological estimators which detect patterns of 'clustering' and 'repulsion' (Cruz-Orive, 1989; Mattfeldt et al. 1993; Mayhew, 1999; Reed & Howard, 1999).

In this study, we test for pore-related differences by quantifying the volume- and surface-weighted volumes of intervillous pores, the theoretical numbers of pores and the spatial arrangements within and between villi and intervillous spaces. Material comprises control placentas and others from groups of diabetic pregnancies varying in insulin-dependence, onset, duration and severity. This provides the

opportunity to test further the efficacy of good glycaemic control at maintaining normal placental morphology.

MATERIALS AND METHODS

Provenance of subjects

A subsample of 25 placentas was selected at random from a larger set collected with relevant background data including maternal age, weight, height, socioeconomic status and reproductive history (Mayhew et al. 1993, 1994; Mayhew & Sisley, 1998). At 8–12 wk of gestation, diabetic pregnant women without hypertension were grouped according to the White classification scheme (White, 1949). Class A diabetic subjects are non-insulin-dependent (type 2) but others are insulin-dependent (type 1, classes B–R) and divided further according to onset (class B: after 20 y of age; C: 10–20 y; D: 0–10 y), duration (B: less than 10 y; C: 10–20 y; D: more than 20 y) and presence of complications (class F: complicated by nephropathy; R: by proliferative retinopathy).

Individual insulin dosages were recommended for class B–R subjects in order to maintain good control (mean blood glucose level: about 6 mmol/l; haemoglobin A_{1c}: less than 7% of total maternal haemoglobin). For this study, subjects were finally grouped as follows: A (n = 7), B + C (n = 4) and D + F + R (n = 6). Subjects were admitted to hospital before term when physical and other examinations were continued. Control placentas (n = 8) were obtained from healthy women who completed uncomplicated pregnancies and were of similar age, body weight, height, parity, gravidity and smoking habits. All neonates were female (to avoid possible confounding effects of sex), showed no evidence of congenital malformations and were delivered after 37 wk of gestation.

Placental sampling

To minimise the relatively large placental transfusion which can occur into neonates of diabetic women (Klebe & Ingomar, 1974), cords were ligatured and cut immediately after delivery. After removing blood coagula and membranes, organs were weighed before immersion in formalin for at least 72 h. Full-depth samples of tissue were taken by systematic random sampling (Gundersen & Jensen, 1987) and embedded in wax blocks after physical randomisation of tissue orientation (Stringer et al. 1982). Five randomly chosen blocks from each organ were cut at a nominal

section thickness of 4 μm , mounted on glass microslides and stained by the Masson trichrome method (Bancroft & Cook, 1984). Working at different magnifications, microscopical fields from sections were selected in a systematic random fashion using an Olympus BH2 microscope with a modified stage equipped with x,y-axis step motors. Final magnifications were calibrated using stage micrometer scales as external standards.

Stereological estimations

A porous medium is one containing connected spaces or interstices. However, the pores may vary considerably in size and number and, in consequence, a proper description of porosity must take pore volume and pore size into account. Therefore, the present study describes porosity in terms of the global volume of all pores, pore size (expressed as a volume-weighted, v_v^* , and as a surface-weighted, v_s^* , star volume) and pore number (expressed in 2 ways by dividing global volume by each type of star volume). Global volumes, total surface areas and star volumes were estimated using design-based stereological methods. Analyses were performed blind by the same individual. Where necessary, estimates were corrected for magnification ($\times 153$) and the effects of tissue processing distortions. These were determined using the diameters of maternal and fetal erythrocytes (measured at $\times 1530$) as internal calibration standards and assuming that tissue shrinkage is uniform and concentric (Burton & Palmer, 1988; Mayhew & Burton, 1988). Previous studies using resin- and paraffin-embedded material have suggested that these corrections improve the comparative worth of stereological estimations.

(a) *Global volume and surface areas.* The total volumes (in cm^3) of villi, intervillous space and perivillous fibrin-type fibrinoid (Frank et al. 1994; Lang et al. 1994) in each placenta were estimated by multiplying each volume density by the corresponding fresh placental volume (calculated from trimmed placental weight and tissue density). Volume densities for villi and intervillous space were estimated as part of the analysis of 3D spatial arrangements (see (e) below) using the point counting principle (Mayhew, 1991). Surface densities of villi within placenta were monitored because surface-weighted star volumes (see (c) below) are influenced by changes in total villous surface area. Surface densities were determined by intersection counting (Mayhew, 1991) and then converted to absolute surfaces (m^2) per placenta.

(b) *Volume-weighted star volumes.* This volume, v_v^* , offers a convenient way of describing the mean

sizes of arbitrary spaces such as the branching villous trees and intervillous pores (Karimu and Burton, 1993; Mayhew & Wadrop, 1994; Lee & Mayhew, 1995; Mayhew & Sisley, 1998). It defines the volume of all parts of a space which are visible when viewed in all directions from a given point within it, the mean being simply the average taken over a set of points randomly sampled within the space (Gundersen & Jensen, 1985). In this study, estimates of v_v^* for intervillous pores were obtained by measuring point-sampled intercept lengths identified by projecting microscopical fields of view onto a set of test points drawn on a sheet of paper laid flat on the workbench. From each test point, a set of 4 straight lines radiated in systematic random directions. When a point fell on the intervillous space, the lengths of lines radiating from this point to surrounding surfaces were measured. This was repeated for all randomly-sampled test points. The surfaces at the ends of intercepts belonged usually to villous trophoblast but occasionally to deposits of perivillous fibrinoid. This definition of 'pore' differs slightly from that adopted in our previous studies (see Mayhew & Wadrop, 1994). On average, 200 intercept lengths, spread over about 15 random fields (3 fields per slide and 5 slides), were measured per placenta. Thereafter, each intercept length was raised to the third power and v_v^* (in μm^3) estimated from the mean of the cubed intercept lengths multiplied by the constant $\pi/3$ (Gundersen & Jensen, 1985).

(c) *Surface-weighted star volumes.* This volume, v_s^* , is related to volume-weighted volume but represents the mean volume of all parts of a space which are visible when viewed in all directions from points on its boundary surfaces (Reed & Howard, 1998). Again, the mean is averaged over a random sample of points. Estimates for intervillous pores were obtained by measuring intersection-sampled intercept lengths generated by projecting fields of view onto a set of test lines so that the encounters between lines and boundary surfaces (maternal aspect of villous trophoblast) were isotropic uniform random in 3D (Karlsson & Cruz-Orive, 1997; Reed & Howard, 1998). From each point (intersection site), the length of test line radiating to surrounding surfaces was measured and this was repeated for all randomly-sampled intersections. Again, roughly 200 intercept lengths on 15 random fields were measured per placenta. Intercept lengths were raised to the third power and v_s^* (in μm^3) estimated from the mean of the cubed intercept lengths multiplied by $2\pi/3$ (Reed & Howard, 1998).

(d) *Number estimations.* From estimates of the global volumes of intervillous spaces and pore star

volumes, numbers of star volume units per placenta were calculated. In other studies (Mayhew & Wadrop, 1994; Lee & Mayhew, 1995), these numbers have provided rough but useful indications of relative intervillous porosity. They do not represent absolute numbers of intervillous pores but only the theoretical numbers of star volume units which could be contained within the global volumes.

(e) *Analysis of pair correlation functions.* The randomness or independence of 3D spatial arrangements between structures can be examined stereologically using second-order methods (Cruz-Orive, 1989). A useful approach is to quantify pair correlation functions, $g[r]$, by analysing the chance encounters between tissue ingredients and dipole test probes (Mattfeldt et al. 1993; Reed & Howard, 1999). Recent applications include analysing spatial arrangements in control and malignant epithelia (Mattfeldt et al. 1993), pulmonary alveoli (Reed & Howard, 1999) and parotid acinar cells (Mayhew, 1999).

A dipole is merely 2 points which, for convenience, can be taken as the 2 ends of a straight line of known length (Reed & Howard, 1999). When tissue images are superimposed on a test lattice made up of sets of dipole probes, the nature of the tissue ingredient hit by each point of each dipole can be recorded. By altering dipole length, clustering (hypodispersion) and repulsion (hyperdispersion) within and between ingredients can be examined at different distances. For present purposes, a test lattice bearing 5 parallel straight lines was drawn on white card to serve as a set of linear dipole probes (Reed & Howard, 1999). Each line was divided into 16 equidistant intervals, giving 17 test points per line and 85 points per lattice. Fields of view from a total of 5 sections per placenta were projected onto this lattice (giving 425 points per placenta). The lattice interval corresponded to $65.4\text{ }\mu\text{m}$ on the specimen scale. This distance was selected because it offers a sensible starting distance with respect to the sizes of villi and intervillous pores. In short, both end points of a dipole probe of class size $r = 1$ (equivalent to $65.4\text{ }\mu\text{m}$) have chances of being included within the same villous or intervillous profile.

Microscopical fields of view were projected onto the lattice so as to be random in position and orientation. Next, the nature of the component underlying each test point was noted and all of this information was recorded on a matrix with 17 columns and 25 rows (i.e. 425 cells). Within the cells of this matrix, each test point was coded as 1 (intervillous space), 2 (villus) or 3 (residuum). Analysis of completed matrices was undertaken using software (provided by Dr MG Reed, University of Liverpool) run on a PC in a DOS

window in Windows95. To estimate volume densities and pair correlation functions, distances between dipole pairs of lattice test points were divided into 10 classes ranging from $r = 0$ ($0\text{ }\mu\text{m}$) to $r = 10$ ($654\text{ }\mu\text{m}$). Higher distances were unnecessary because pilot studies indicated that group mean values of $g[r]$ did not alter appreciably from those found at $r = 10$. Moreover, larger dipole distances reduced the precision of estimation. Values of *set* covariances $C[r]$ at each dipole distance were calculated by summing the number of dipoles whose points both hit the *same* component (either intervillous pores, $C[r]_{11}$, or villi, $C[r]_{22}$), and dividing by the total whose points both hit the placental reference space. At $r = 0$, the set covariances are equal to the corresponding component volume densities, V_{v1} and V_{v2} . Pair correlation functions for a single component were estimated by dividing each set covariance by the square of the corresponding volume density, V_{v1} or V_{v2} . If components are randomly (independently) distributed in space, the expected value of $g[r]$ is 1. This value therefore provides a convenient internal 'null hypothesis' for testing for clustering ($g[r] > 1$) or repulsion ($g[r] < 1$) within and between components.

For estimating *cross* covariances, $C[r]_{12}$, at each dipole distance, numbers of dipoles whose points hit villi *and* intervillous space were counted and divided by the total hitting the reference space. Pair correlation functions for 2 components were estimated from cross covariances by dividing by the product of both volume densities ($V_{v1} \times V_{v2}$). Again, $g[r] = 1$ for a random (independent) distribution of 2 components (Mattfeldt et al. 1993).

Model-based calculations of pore hydraulic dimensions

The haemodynamic properties of vascular spaces differ according to whether they form a porous medium or a system of tubes (Dullien, 1979; Peeters & Buchan, 1989). To calculate effective viscosities and shear rates, we would need to know the numbers of cotyledons per placenta, the maternal blood flows and the hydraulic diameters of intervillous spaces in each placenta. We can make rough calculations of the latter by treating pores, for convenience, as a homogeneous set of right circular cylinders. The hydraulic (= cross-sectional) diameter of such cylinders is defined as $4 \times V/S$ (Dullien, 1979) where, in the present situation, V equates with the global volume of the intervillous space and S with its total boundary (villous) surface area. To fully characterise such a hypothetical pore, the mean cylinder length is

also of interest. One approach to estimating this 'hydraulic length' is to use the volume-weighted mean volume of the average pore and the hydraulic diameter to calculate the length of a cylinder of equivalent volume.

Statistical analyses

Means and standard errors of means (S.E.M.) were calculated for all groups. Between-group comparisons were made using 1-way analyses of variance (Sokal & Rohlf, 1981) with Unistat commercial software for MS Windows (version 4.53c, Unistat Ltd, London). Where appropriate, post hoc testing with Student's *t* test was undertaken. The null hypothesis was rejected if the probability level, *P*, was less than 0.05.

For analysing spatial distributions within and between groups, values of $g[r]$ were plotted against dipole distance with the baseline $g[r] = 1$ as an internal test of departures (clustering or repulsion) from randomness. Group means and S.E.M. were used to calculate 95% confidence intervals from Student's *t* values for the appropriate degrees of freedom (Mattfeldt et al. 1993). To compare diabetics with controls, values of $g[r]$ in different diabetic groups were plotted with the control confidence intervals. Statistics and graphs were handled using the Unistat statistical package.

RESULTS

Present subsamples reproduced the findings of the larger study in which blood glucose and glycated haemoglobin levels were well-controlled, the respective median (average) values being about 6 mmol/l of glucose (6% glycated haemoglobin) in diabetic groups A and BC and 9 mmol/l of glucose (7% glycated haemoglobin) for group DFR. For control mothers, the mean (S.E.M. characteristics were: age 28.8 (2.24) y,

weight 77.0 (6.74) kg, height 160 (2.1) cm, parity 1.7 (0.33) and total haemoglobin 7.85 (0.45) mmol/l. Their neonates were characterised as follows: weight 2.97 (0.20) kg, crown-rump length 50.7 (1.4) cm and cord haemoglobin 9.55 (0.23) mmol/l. The mean placental volume was 420 (42.4) cm³. Except for cord haemoglobin levels (higher in diabetic subjects), apparent differences in maternal, neonatal and placental characteristics between groups were not significantly different.

Global volumes and surface areas

Results are provided in Table 1. In the control group, the average placenta contained 198 (21.4) cm³ of villi with a total surface of 8.47 (0.77) m². It also possessed an intervillous space amounting to 171 (22.8) cm³ and containing 8.48 (0.84) cm³ of fibrin-type fibrinoid. These estimates did not differ significantly between groups when tested using one-way analyses of variance and, again, this reproduces the findings of the larger investigation. However, post hoc testing suggested that class A (type 1) diabetics possessed greater volumes and surface areas of villi than controls but these variables were not significantly different from values found in the other (type 2) diabetic groups.

Star volumes and numbers of star volume units

In the average control placenta, the intervillous space harboured about 66 (15) million pore units with a mean volume-weighted star volume of $3.35 (0.58) \times 10^6 \mu\text{m}^3$. The space and its boundary villous surface also contained approximately 187 (40) million pore units with a mean surface-weighted star volume of $1.64 (0.71) \times 10^6 \mu\text{m}^3$. Apparent between-group differences in star volumes and theoretical numbers of pores were not significant.

Table 1. Villous and intervillous volumes, star volumes and theoretical numbers of star volume units

Variable	Controls	Diabetic A	Diabetic BC	Diabetic DFR
(A) Villi				
Global volume (cm ³)	198 (21.4)	291 (39.8)	273 (35.2)	258 (18.3)
Total surface (m ²)	8.47 (0.77)	12.0 (1.59)	11.1 (1.58)	9.54 (0.73)
(B) Intervillous space				
Global volume (cm ³)	171 (22.8)	214 (24.5)	200 (26.7)	186 (15.8)
Fibrinoid volume (cm ³)	8.48 (0.84)	16.9 (4.04)	12.1 (5.32)	9.78 (2.02)
v_v^* volume ($\mu\text{m}^3 \times 10^6$)	3.35 (0.58)	2.35 (0.43)	2.20 (0.80)	1.96 (0.26)
Pore number for v_v^* ($\times 10^6$)	66.2 (15.0)	107 (17.6)	138 (50.6)	108 (23.1)
v_s^* volume ($\mu\text{m}^3 \times 10^6$)	1.64 (0.71)	1.00 (0.22)	0.92 (0.34)	0.58 (0.11)
Pore number for v_s^* ($\times 10^6$)	187 (40.2)	313 (90.6)	329 (126)	414 (105)

Values are group means (S.E.M.).

Table 2. Derived (model-based) quantities characterising pores of the intervillous space

Variable	Controls	Diabetic A	Diabetic BC	Diabetic DFR
Hydraulic diameter (μm)	80 (5.7)	74 (7.6)	75 (13.2)	78 (4.8)
Hydraulic length (μm)	704 (146)	603 (114)	590 (194)	411 (58.5)

The calculations rely on the simplifying assumption that pores can be modelled as right circular cylinders. Values are group means (S.E.M.).

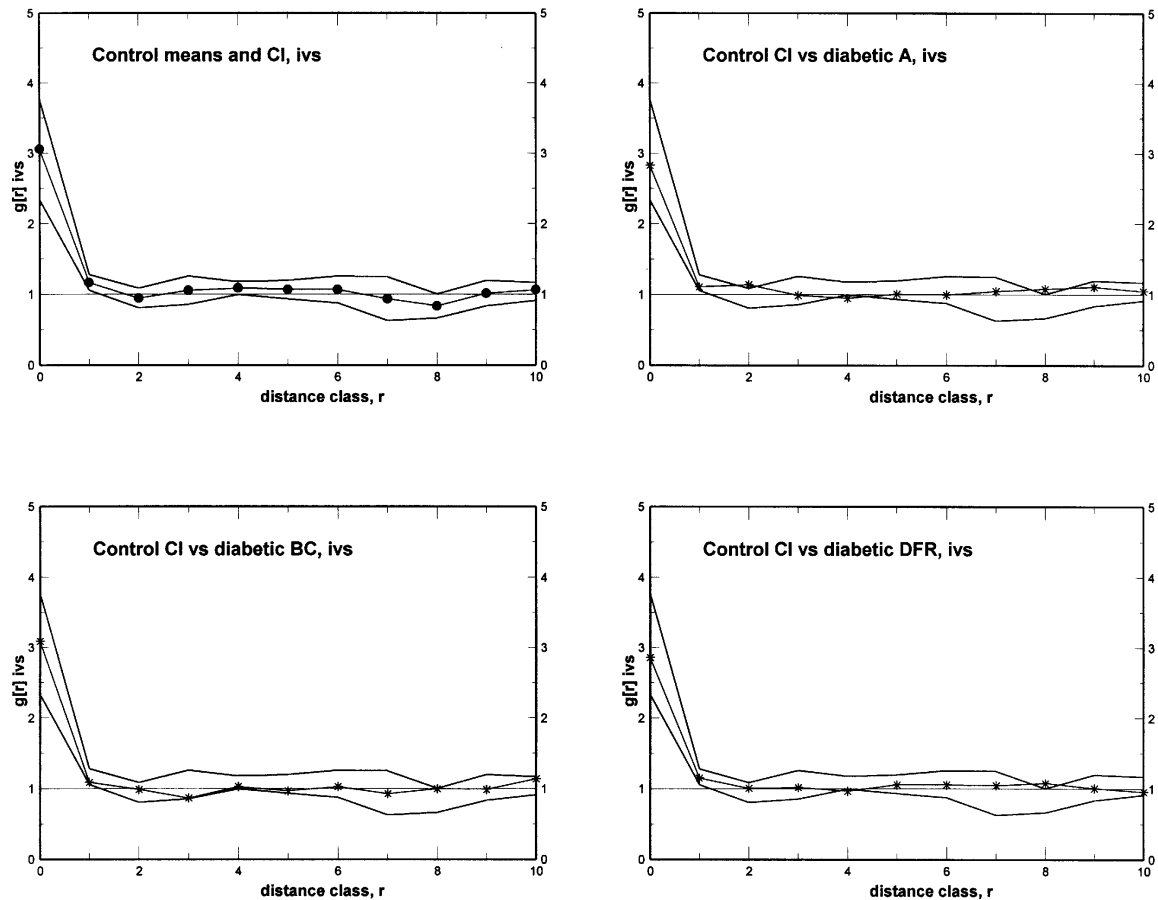


Fig. 1. Relationships between pair correlation function and dipole distance for intervillous pores in control and 3 groups of diabetic placenta. To facilitate comparisons, plots show group means together with the control 95% confidence intervals (CI). Horizontal lines indicate the expected value of $g[r]$ for a 'random' arrangements of pores. In all plots, pores are clustered at short distances but neither clustered nor dispersed at larger distances.

The hydraulic dimensions of cylindrical pores

On the simplifying assumption that pores can be modelled as cylinder elements of constant diameter and length, the group mean hydraulic diameters and lengths are given in Table 2. The mean diameter for control placentas was 80 (5.7) μm and the corresponding values for diabetics were 74 (7.6) μm for group A, 75 (13.2) μm for group BC and 78 (4.8) μm for group DFR. Apparent differences between groups were not significant and this is consistent with the failure to detect differences in star volume estimates. Using the same model, and corresponding volume-weighted star volume estimates, these diameters yield

cylinder lengths of 704 (146) μm for controls, 603 (114) μm for group A diabetics, 590 (194) μm for group BC and 411 (58) μm for group DFR. Again, apparent differences between groups did not attain statistical significance.

Spatial relationships of villi and intervillous pores

Findings are represented graphically in Figures 1–3 in which pair correlation functions, together with control 95% confidence intervals, are plotted against dipole distance.

For intervillous space in control placentas (Fig. 1), the pattern of $g[r]$ at different dipole lengths indicated

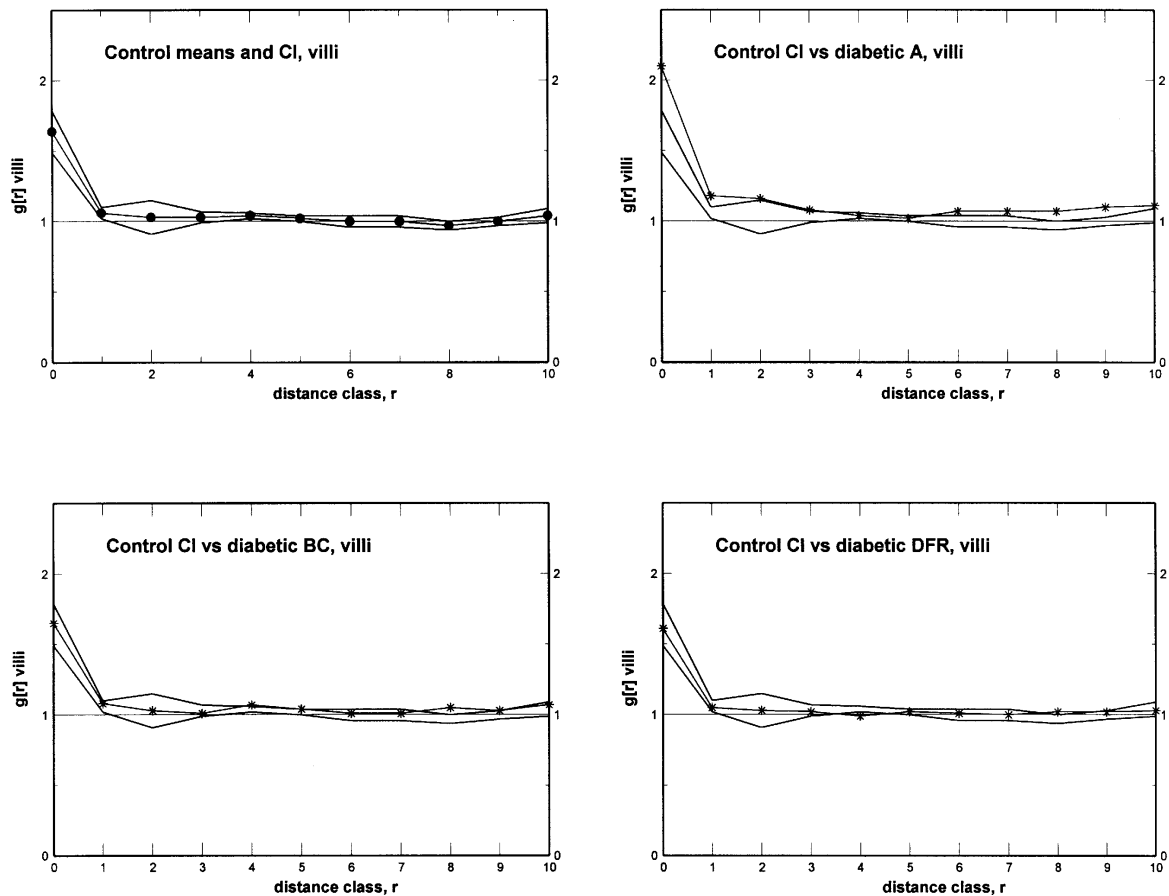


Fig. 2. Relationships between pair correlation function and dipole distance for villi in control and diabetic placentas. Plots show group means together with control 95% confidence intervals (CI) and horizontal lines indicate the expected value of $g[r]$ for 'random' arrangements of villi. In all plots, villi cluster at short distances but tend not to be clustered or dispersed at larger distances.

relatively tight clustering at short distances (below about 130 μm but, particularly, below 65 μm). Beyond 130 μm , the spatial relationships are consistent with those expected for a random (independent) distribution (i.e. $g[r] = 1$). Basically the same patterns were found in all 3 groups of diabetic placentas whose mean values of $g[r]$, for the most part, fell within the confidence intervals of the controls. However, values in class A diabetics tended to be larger than those in other groups at dipole distances of 130 μm (class $r = 2$) and 520 μm (class $r = 8$). This is probably due to chance variation.

In control placentas, the values of $g[r]$ for villi suggested relatively looser clustering below about 65 μm and a random arrangement at larger distances (Fig. 2). This basic pattern was maintained in diabetic placentas but the clustering at smaller distances, and at roughly 590 μm (class $r = 9$), was slightly greater in group A diabetics. The former is consistent with relatively greater villous surfaces and volume densities.

When relationships between intervillous spaces and villi were examined together, $g[r]$ values in control and

diabetic placentas were similar to those expected for spatial independence (Fig. 3). Again, values at smaller distances (less than 130 μm) tended to be less homogeneous in class A diabetics than those in other diabetic groups.

DISCUSSION

Although based on more modest sample sizes, the present subsamples succeeded in reproducing the major findings of the larger studies (Mayhew et al. 1993, 1994). Here, we examined the sizes of intervillous pores by stereological design-based methods (estimating volume- and surface-weighted star volumes) and a model-based approach (calculating the hydraulic diameters and lengths of uniform cylinders). In addition, we analysed spatial relationships within and between the 2 main parenchymal compartments of the human placenta (villi, intervillous space) using second-order stereology (estimating set and cross pair correlation functions). The findings were internally consistent and demonstrated

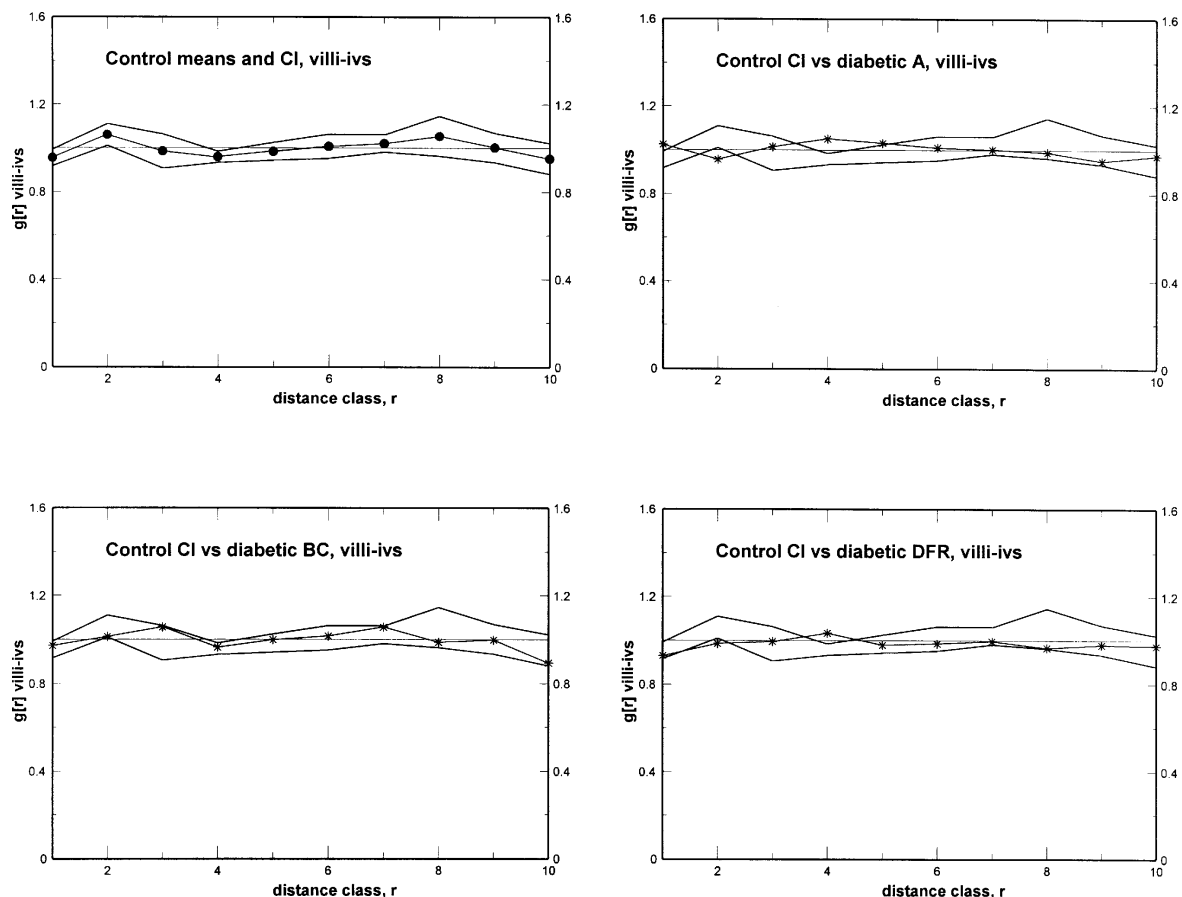


Fig. 3. Relationships between pair correlation function and dipole distance for villi and intervillous pores in control and diabetic placentas. Plots show group means together with control 95% confidence intervals (CI) and horizontal lines indicate the expected value of $g[r]$ for a 'random' arrangement. The plots suggest that, in all groups, villi and pores neither cluster nor repel each other.

that, for the most part, functionally relevant spatial features and relationships are similar in control and well-controlled diabetic placentas regardless of the duration and severity of maternal diabetes mellitus. Departures from control values were seen in class A (type 1) diabetic placentas for the global volumes and surface areas of villi. Departures were also seen for pair correlation functions of intervillous spaces and villi but only at the shortest (0–130 μm) and longer (520–590 μm) dipole distances. The latter discrepancies are likely to be attributable to random errors rather than real features of class A diabetics.

Total volumes and surface areas

Although not in unanimous agreement, previous studies on diabetic placentas from different White classes have tended to find more expansive villous surface areas (Singer et al. 1981; Teasdale, 1981, 1985; Björk & Persson, 1984; Boyd et al. 1986; Teasdale & Jean-Jacques, 1986; Stoz et al. 1988). The present finding of enlarged surfaces in type 1 (insulin-

dependent), but not type 2 (non-insulin-dependent), diabetic pregnancies is consistent with apparent, though not significant, increases in an A+B+C grouping of placentas in a study based on larger samples (Mayhew et al. 1994). A marked increase in villous surface area might be expected to affect estimates of the surface- and volume-weighted star volumes of intervillous pores as well as the pair correlation functions for villi and intervillous pores. Whilst no significant differences in star volume estimates were detected in any of the present diabetic groups, values appeared to be smaller than in controls and this was also true of samples of BC and DFR placentas taken in a recent investigation of volume-weighted pore volumes (Mayhew & Sisley, 1998). Intervillous $g[r]$ values appeared to be greater at short and long distances suggesting that pores may be smaller or more clustered at these distances. This is worth noting because an enlarged villous compartment would be expected to reduce pore size and, on a cylinder model, this might involve both 'diameter' and 'length' (both of which seem to decline in diabetic pregnancies).

There were no significant differences in the global volumes of intervillous spaces or deposits of perivillous fibrin-type fibrinoid within them. However, diabetic placentas were far more variable in terms of their fibrin-type fibrinoid content suggesting that the fibrinoid may have a different spatial arrangement. This possibility requires further investigation. Other things being equal, a constant volume of intervillous space implies that maternal flow rates and transit times are likely to be preserved in well-controlled diabetic pregnancies. Interestingly, trends towards increases in global volumes of the intervillous space have been reported in classes A, B and C diabetics (Teasdale, 1981, 1983, 1985). Where glucose levels are managed less well, it is known that flow rates through the intervillous space may be reduced (Nylund et al. 1982). Deposition of fibrin-type fibrinoid in the intervillous space may be due to the mechanical effects of blood flow or to stasis of blood coupled with clotting and may have a strategic role in adapting maternal blood flow away from poorly perfused areas (Kaufmann & Burton, 1994). This may, in turn, lead to local adjustments of villous arborisations including cytotrophoblast proliferation and syncytial knot formation (Jones & Fox, 1976; Björk & Persson, 1982). It is also known that perivillous fibrinoid may be associated with regions of trophoblast which are de-epithelialised or contain apoptotic nuclei (Nelson, 1996; Huppertz et al. 1998).

Hydraulic dimensions

In a porous medium, the type of blood flow and accompanying shear forces differ from those in vascular tubes (Dullien, 1979; Peeters & Buchan, 1989). Nevertheless, the concept of cylindrical vascular elements is a useful one for comparative purposes. Our calculations of the hydraulic diameters of intervillous spaces indicate that the control value is maintained in diabetic placentas. This demonstrates internal consistency in our data sets because we detected no significant differences in the design (rather than model)-based estimates of pore star volumes. In addition, the predicted hydraulic lengths of cylindrical pores found in control organs were not altered significantly in diabetics. In short, if pores were modelled as circular cylinders (a convenient but gross oversimplification), they would have a mean diameter of about 80 μm and a length of about 700 μm . These values may be of practical significance since analysis of pair correlation functions for intervillous spaces indicated that there was significantly tighter clustering

($g[r]$ values > 1) in class A diabetics at shorter ($\sim 130 \mu\text{m}$) and larger ($\sim 520 \mu\text{m}$) dipole distances. The implication is that differences in pore size could be contributing to local perturbations of spatial arrangements. This idea could be tested by examining larger numbers of placentas.

Volume and surface weighted star volumes

The use of v_v^* to determine the sizes of intervillous pores has benefits and disadvantages. Although providing a useful way of realising the concept of intervillous porosity (Schmid-Schönbein, 1988), v_v^* is a statistically noisy variable. In previous studies on placentas during normal gestation (Mayhew & Wadrop, 1994) and pregnancy at different altitudes (Lee & Mayhew, 1995), organ-to-organ coefficients of variation (CV) in the range 49–298 % of group means have been found for the v_v^* of intervillous pores. Present values (CV 33–73 %) lie at the lower end of this range and more closely resemble ranges seen in other samples taken from control and diabetic placentas (CV 69–92 %; Mayhew & Sisley, 1998). Unfortunately, present results suggest that v_s^* may be even noisier (CV 45–123 %) than v_v^* (33–73 %).

Noisiness of these estimators stems from the fact that intervillous pores are point-sampled local regions of arbitrary space. Therefore, pore v_v^* depends on a variety of factors including the number, size and spatial (lobular) arrangement of villous trees within the intervillous space, the presence of fibrinoid deposits, the periodical fountain-like spurting of maternal blood from openings of uterine spiral arteries and the amount of blood retained when the placenta detaches from the uterine wall. Similar factors influence surface-weighted star volumes which, by definition, further depend on villous surface area.

Although an earlier study also failed to find any significant differences between controls and diabetics in values of v_v^* (Mayhew & Sisley, 1998), both investigations have revealed apparent decline in diabetics. Clearly, resolution of this possibility requires analysis of larger samples.

The rationale for measuring v_s^* is that this variable is influenced more directly by differences in total villous surface area. By focusing on the relationship between villous surface and pore volume, the relevance to issues associated with transport across the villous surface is more obvious. Unfortunately, like volume-weighted star volume, v_s^* is a noisy variable (see also Reed & Howard, 1998). Present results suggest that it may be even noisier and so its

application in future studies will need to balance this imprecision against the potential benefits of being able to characterise pore sizes more comprehensively.

Spatial relationships

Unlike star volumes, pair correlation functions exhibited relatively little noise and little variation between placentas. Essentially, this is due to the fact that it is a basic architectural pattern which is being expressed (rather than sizes of ingredients which contribute to that pattern) and this has a strong self-similarity within and between groups of placentas. Villi and intervillous pores both displayed clustering at shorter dipole distances but were essentially randomly arranged at larger distances. Similar patterns within single compartments have been observed in lung alveoli (Reed & Howard, 1999) and the rough endoplasmic reticulum and mitochondria of parotid acinar cells (Mayhew, 1999). The relationships between villi and intervillous pores were also essentially random. These findings must not be misinterpreted as implying that placental parenchyma is randomly arranged. Clearly, the intimate associations between the maternal vascular bed and villi are crucial to successful functioning and are not random in the local sense. Instead, the findings merely indicate that once outside the local spaces encompassed by the shorter distances, there is no evidence of dependence or of a consistent pattern of component clustering or dispersion.

In the main, the above arrangements were maintained in diabetic placentas. Minor departures from control values for intervillous pores were seen in class A diabetics at short and long distances and these perturbations might be indicative of subtle changes in pore size (see above) and volume density.

Concluding remarks

Hitherto, the observed pattern of structural change in maternal diabetes mellitus has been one in which adaptations are focused on the fetal side of the placenta (Mayhew et al. 1993, 1994; Mayhew & Sisley, 1998). These must be seen as positive adaptations to maintain fetal oxygen supplies but, nevertheless, the fetus suffers chronic hypoxia as evidenced by the elevated levels of erythropoietin and cord haemoglobin (Widness et al. 1981, 1990). In contrast to these downstream responses, relatively little attention has been accorded to possible changes in spatial arrangements on the maternal side.

In our hands, the total volume of intervillous space, the volume of perivillous fibrin-type fibrinoid and the 2 star volume estimators of intervillous pore size were maintained at normal values in placentas from well-controlled diabetic pregnancies. Given that uteroplacental blood flows are decreased in diabetic pregnancies (Nylund et al. 1982), the implication is that either blood flows are normalised by good metabolic control or they are reduced by factors other than smaller pore sizes or excessive fibrin deposition. Post hoc tests revealed a small number of significant differences involving the non-insulin-dependent (White class A) diabetics. These findings serve to emphasise the importance of providing proper pre-pregnancy counselling and treatment to these women and closely following them and their fetuses and neonates (Hellmuth et al. 1997).

In all groups of diabetics, metabolic control was reasonably tight but the apparent changes in certain placental variables noted here may serve as predictors of the likely consequences of poor control. These would seem to include expanded villous surface areas and global intervillous volumes, reduced pore sizes and more clustered spatial associations. These predictors would seem to fit the structural and blood flow alterations reported previously (Singer et al. 1981; Teasdale, 1981, 1983, 1985; Nylund et al. 1982; Björk & Persson, 1984; Boyd et al. 1986; Teasdale & Jean-Jacques, 1986; Stoz et al. 1988) including the increase in cytotrophoblast hyperplasia (Jones & Fox, 1976). Provided there is no hypoxia-related impairment of cytotrophoblast fusion into syncytiotrophoblast (Alsatt et al. 1996), the natural differentiation within syncytium (Huppertz et al. 1998; Mayhew et al. 1999) might be expected to contribute to the expansion of villous surface area (Mayhew & Simpson, 1994).

To conclude, it appears that fetal hypoxia persists in well-controlled diabetes mellitus despite the preservation of essentially normal placental morphology on the maternal side and adjustments on the fetal side (see also Mayhew et al. 1994; Mayhew & Sisley, 1998). This strengthens the case that differences are mainly due to the metabolic disturbances associated with diabetes (Desoye & Shafrir, 1996).

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